

ELEVATED ATMOSPHERIC CARBON DIOXIDE EFFECTS ON SORGHUM AND SOYBEAN NUTRIENT STATUS¹

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ABSTRACT: Increasing atmospheric carbon dioxide (CO₂) concentration could have significant implications on technologies for managing plant nutrition to sustain crop productivity in the future. Soybean (*Glycine max* [L.] Merr.) (C₃ species) and grain sorghum (*Sorghum bicolor* [L.] Moench) (C₄ species) were grown in a replicated split-plot design using open-top field chambers under ambient (357 $\mu\text{mol/mol}$) and elevated (705 $\mu\text{mol/mol}$) atmospheric CO₂. At anthesis, leaf disks were taken from upper mature leaves of soybean and from the third leaf below the head of sorghum for analysis of plant nutrients. Leaf greenness was measured with a Minolta SPAD-502 chlorophyll meter. Concentrations of chlorophylls *a* and *b* and specific leaf weight were also measured. Above-ground dry matter and seed yield were determined at maturity. Seed yield of sorghum increased 17.5% and soybean seed yield increased 34.7% with elevated CO₂. There were no differences in extractable chlorophyll concentration or chlorophyll meter readings due to CO₂ treatment, but meter readings were reduced 6% when sorghum was grown in chambers as compared in

1. Contribution of USDA-ARS in cooperation with the Alabama Agricultural Experiment Station.

the open. Leaf nitrogen (N) concentration of soybean decreased from 54.5 to 39.1 g/kg at the higher CO₂ concentration. Neither the chambers nor CO₂ had an effect on concentrations of other plant nutrients in either species. Further work under field conditions is needed to determine if current critical values for tissue N in crops, especially C3 crops, should be adjusted for future increases in atmospheric CO₂ concentration.

INTRODUCTION

Although the causes of increased atmospheric CO₂ and the effects of this increase on global climate are hotly debated within the scientific and political communities, the fact that atmospheric CO₂ levels are increasing is irrefutable (Nefiel et al., 1985; Keeling et al., 1989). The concentration of CO₂ in the atmosphere has increased approximately 62 µmol/mol during the last century (Overdieck et al., 1988) and modellers predict that by the end of the next century atmospheric CO₂ will essentially double from current levels (Edwards et al., 1984; Bolin et al., 1986).

As CO₂ levels rise, plant growth and agricultural yields will increase as a result of increased rates of photosynthesis and increased water use efficiency (Kimball, 1983; Rogers and Dahlman, 1993). Elevated levels of CO₂ increase plant growth in C3 species like cotton (*Gossypium hirsutum* L.) and soybean by increasing leaf area and photosynthesis per unit leaf area, while in C4 plants like corn (*Zea mays* L.) and sorghum, increased growth is a result of lowered stomatal conductance and increased water-use efficiency (Rogers et al., 1983a).

Little research has focused on plant nutrient interactions in relation to increased atmospheric CO₂ (Rogers and Dahlman, 1993). What research has been done has been performed with containerized plants in greenhouses or growth chambers (Wong, 1979; Sionit et al., 1981; Patterson and Flint, 1982; Sionit, 1983; Peet et al., 1986; Overdieck et al., 1988; Hocking and Meyer, 1991; Overdieck, 1993). In these artificial environments, the general finding has been that increased plant growth under elevated CO₂ results in reduced plant tissue nutrient concentrations (Hocking and Meyer, 1985; Larigauderie et al., 1988; Overdieck et al., 1985; Hocking and Meyer, 1991). This limited research suggests that future increases in atmospheric CO₂ levels will have an impact on plant

nutrient concentration analyses and interpretations used to manage soil fertility and plant nutrition in agricultural production systems.

The purpose of this study was to determine the effect of elevated atmospheric CO_2 on leaf tissue nutrient concentrations and yield of two crop species, soybean (C3 species) and grain sorghum (C4 species) grown under field conditions. The leaf tissue sampling was done at a commonly recommended stage of growth for diagnosis of plant nutritional problems, i.e. anthesis for grain sorghum and full bloom-beginning pod set for soybean. We also determined CO_2 effects on measurement of leaf chlorophyll using a hand-held meter which can be used to evaluate plant N status (Piekielek and Fox, 1992; Wood et al., 1992, Reeves et al., 1993).

MATERIALS AND METHODS

This study was conducted on an outdoor soil bin located at the USDA-ARS National Soil Dynamics Laboratory in Auburn, AL. The bin is uniformly filled with Blanton loamy sand topsoil (loamy, siliceous, thermic Grossarenic Paleudult). The bin is 2 m deep, 7 m wide, and 7 m long. The bottom of the bin is covered with sand and gravel and is tile drained.

Soybean, 'Stonewall', and grain sorghum, 'Savanna 5', were planted in 76 cm rows oriented across the width of the bin on 2 June 1993. Soybean and sorghum were chosen as test crops to provide a C3 (soybean) and C4 (sorghum) crop species, as few studies have been conducted where both C3 and C4 species were compared directly when grown under elevated CO_2 . Plants were thinned to a uniform density of 30 plants/m² for soybean and 26 plants/m² for sorghum. The two crops were arranged as main plots in a split-plot design of three replications within the bin. Within each crop species in each replication, two open-top PVC plastic covered chambers and a fully open chamber (structural aluminum anchoring without any PVC enclosure) were arranged as subplots.

The chambers were 3 m in diameter and 2.4 m high. Two levels of CO_2 concentration (ambient and twice ambient) were maintained during the growing season in the open-top chambers. Carbon dioxide concentrations were continuously monitored using a time-shared sampling manifold with samples drawn through solenoids to an infrared CO_2 analysis. The CO_2 monitoring-dispensing

system was computer controlled via a continuous data acquisition system. Carbon dioxide was supplied from a 12.7 Mg liquid CO₂ receiver through a high volume dispensing manifold and added to the chambers by injection into plenum boxes. Air was dispensed into each chamber through the bottom half of each chamber cover which was doubled-walled. The inside wall was perforated with 2.5 cm diameter holes to serve as ducts to distribute air uniformly into the chamber. The CO₂ exposure system is similar to that described by Rogers et al. (1983b). Average CO₂ concentrations (measured over 23,016 samplings) maintained during the course of the study were. 357.4 ± 0.1 (S.E.) $\mu\text{mol CO}_2/\text{mol}$ in the ambient chambers and 705 ± 0.3 (S.E.) $\mu\text{mol CO}_2/\text{mol}$ in the twice ambient chambers.

The fully open chamber (aluminum anchor-ring only) provided a control to test the effect of the open-top PVC chambers per se versus the effects of CO₂ on plant response. The average CO₂ concentration during the study in the open chamber was 357.5 ± 0.1 (S.E.). The open chamber within each main plot (plant species) was not randomly located within the main plot area as were the two CO₂ level treatments. This was necessary because of size restrictions of the chambers and to minimize any intrashading effect of the chambers.

Soil test results 83 days prior to planting, at planting, and at the end of the growing season are shown in Table 1. Fertilizer and lime additions to the soil bin are also shown in Table 1. The initial low fertility status of the Blanton soil provided a baseline to better evaluate the effects of elevated CO₂ on soil conditions as the study continues in the long-term. Lime and fertilizer inputs were adequate to prevent nutrient deficiencies.

A low volume sprinkler micro-irrigation system was used to water stress during periods of insufficient rainfall. During the growing season, a total of 146 mm of water was applied in eight applications. Rainfall distribution was good during the study period and totaled 401 mm

On 4 August 1993, 1 cm diameter leaf disks were collected from the upper mature leaves of soybean and from the third leaf below the head of sorghum. Soybean was at full bloom-early pod set and sorghum was at anthesis at this time. These growth stages are considered standards for evaluating the nutrient status of these two crops in regards to plant nutrition/soil fertility recommendations (Small and Ohlrogge, 1973; Jones et al., 1991). Two disks from ten individual plants in

TABLE 1. Soil Test Results (0-15 cm depth) and Fertilizer Additions to the Blanton Loamy Sand Used in This Study.

Initial - 10 March 1992 (-83) ^z												
O.M.	C.E.C.	pH	P	K	Ca	Mg	Cu	Fe	Mn	Zn	B	Mo
g kg ⁻¹	cmol _c kg ⁻¹		kg ha ⁻¹				mg kg ⁻¹					
5	2.45	4.7	8	14	56	8	0.2	15.1	4.4	5.9	0.1	0.1
			VL ^r	VL	L	L						
10 June 1992 (+8)												
-	- ^x	5.4	35	84	569	36	0.9	15.5	4.5	1.2	0.3	0.1
			M	M	H	H						
18 February 1993 (+260)												
-	-	6.3	48	61	552	50	0.4	18.1	7.5	1.8	0.2	0.2
			M	M	H	H						
Fertilizer Additions (kg ha ⁻¹) ^w												
Date			Lime	N	P	K	Mg	S	B	Zn	Mo	
2 April 1992 (-61)			3360	-	-	-	-	-	-	-	-	
8 May (-25)			-	-	74	140	5.6	11.2	2.2	3.4	0.07	
4 June (+2)			-	34	-	-	-	-	-	-	-	
2 July (+30)			-	67 ^v	20	-	5.6	11.2	2.2	3.4	0.07	

^z number in parentheses are days in relation to planting.^r VL=very low, L=low, M=medium, H=high soil test rating (Cope et al., 1980).^x - =not determined or not applied.^w sources of nutrients were dolomitic lime, ammonium nitrate, triple superphosphate, muriate of potash, potassium-magnesium sulfate, zinc sulfate, borax, and sodium molybdate.^v N applied to sorghum only.

each treatment were collected. Disks were collected one-third the distance from the tip of the leaves-midway between the midrib and leaf edge of sorghum or between major leaf veins in soybean. Fifteen disks from each treatment were dried at 60°C and ground to pass a. OS-mm sieve. One-half-gram samples from the

disks were ashed in a muffle furnace at 450°C for 4.5 hr. The ash was dissolved in 10 mL 1M HNO₃ and slowly evaporated to near dryness on a hot plate. The remaining residue was dissolved in 1M HCl, heated to boiling, diluted to 100 mL with deionized water, and filtered through a Whatman No. 1 filter paper. Calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), boron (B), and molybdenum (Mo) in the digests were measured by inductively coupled argon plasma spectrophotometry. Carbon and N in the disks were determined with a LECO CHN-600 Analyzer² (LECO Corp., St. Joseph, MI).

In addition to collecting leaf disks, leaf chlorophyll measurements were made using a Minolta SPAD-502 Chlorophyll Meter² (Minolta Camera LTD., Osaka, Japan). The same sampling scheme was used for meter readings as for leaf disks. This provided a total of 20 readings for each treatment plot. Concentrations of chlorophylls *a* and *b* were determined from five leaf disks from each leaf using the procedure of Inskeep and Bloom (1985). Results are reported as total chlorophyll.

An additional ten leaf disks from each plot, sampled as described previously, were dried to a constant mass at 60°C and weighed for determination of specific leaf weight. Specific leaf weight was determined as a measure of leaf thickness because leaf thickness can affect chlorophyll meter readings (Palta, 1990) and one reported effect of elevated CO₂ is to increase leaf expanse and thickness (Rogers et al., 1983c; Thomas and Harvey, 1983).

At maturity, total above-ground dry matter and seed yields were determined from the entire chamber area. All plant material was dried to a constant mass at 60°C. Total biomass and seed yields are reported on a dry weight basis (0 kg/kg moisture).

Treatment effects for each crop species were analyzed separately *using a* randomized complete block model. In addition, because the fully open chamber was not randomized in regards to placement within the main plots (crop species),

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separate analyses of variance for a split plot model were used to determine species X CO₂ and species X chamber interaction effects on response variables. To test species X CO₂ interactions, subplots in the model were ambient (357 µmol CO₂/mol) and twice ambient (705 µmol CO₂/mol) open-top chambers. To test species X chamber interactions, subplots assigned to the model were the ambient open-top chamber and the fully open (aluminum anchor-ring only) structure. Means were separated using protected LSD at the 0.05 level of significance.

RESULTS AND DISCUSSION

Leaf Nutrient Concentrations

Neither plant species, chamber (i.e. comparing the 357 µmol/mol CO₂ concentration versus the fully open chamber) nor elevated CO₂ had an effect on any element other than N. Leaf disk tissue nutrient concentrations other than N, averaged over CO₂ rate, chambers, and crop species, were: Ca 5.3 g/kg, K 14.2 g/kg, Mg 2.2 g/kg, P 2.9 g/kg, Cu 16 mg/kg, Fe 54 mg/kg, Mn 141 g/kg, Zn 32 mg/kg, B 141 mg/kg, and Mo 2 mg/kg. Boron concentrations were in the high range for both crops (Jones et al., 1991), reflecting the addition of B to the soil bin (Table 1). Potassium concentrations were below the sufficiency range for soybean but sufficient for sorghum, and Mg concentration was within the sufficiency range for sorghum and slightly low for soybean (Jones et al., 1991). These sufficiency ranges are based on whole leaf samples. The leaf disks, taken from the middle of the lamina, likely had a higher concentration of elements than would have been measured from entire leaf blades, with the exception of K, which likely had a lower concentration in the disks than would be measured in entire leaves (Jones et al., 1991). Thus, it is likely that K concentrations in plants were not insufficient.

Chamber Effects on Nitrogen

Leaf disk N concentration averaged 56.0 g/kg for soybean and 36.1 g/kg for sorghum (LSD_{0.05} = 0.952). Separate analyses of variance by species indicated that chambers reduced leaf N concentration in soybean but not in sorghum (Table 2), however, the species X chamber interaction was not significant (P<0.10). This was concomitant with a greater reduction in dry weight for soybean grown in the

TABLE 2. Effect of open-top chamber used in CO₂ study on leaf N, chlorophyll concentration, chlorophyll meter reading (SPAD units), total dry matter production and seed yield of sorghum and soybean.

Crop	Chamber	Leaf N (g kg ⁻¹)	Total Chlorophyll (µg cm ⁻²)	SPAD -	Total Dry Matter (kg ha ⁻¹)	Seed Yield (kg ha ⁻¹)	Specific Leaf Weight (g m ⁻²)
Sorghum	yes	36.6 a	38.5 a	46.6 a	7381 b	3802 b	31.07 a
	no	35.6 a	37.3 a	43.8 b	7927 a	4186 a	30.90 a
Soybean	yes	54.5 b	36.1 b	40.0 a	8170 b	2501 b	29.11 a
	no	57.4 a	39.3 a	40.4 a	9279 a	3087 a	25.90 a
LSD _{0.05}		ns ($P \leq 0.10$)	2.47	ns ($P \leq 0.07$)	310	ns ($P \leq 0.12$)	ns ($P \leq 0.31$)

^zLSD for species x chamber interaction (any two means), ns=not significant. Means within a species followed by same letter are not significantly different.

chambers as compared to sorghum (Table 2). Therefore, the reduced N concentration of soybean grown in chambers cannot be attributed to a dilution effect from increased dry matter production. Nitrogen concentrations of leaf disks were within the sufficiency range for sorghum and were on the high end of the sufficiency range for soybean (Jones et al., 1991). but as discussed for other elements, concentrations of N in leaf disks would be expected to be slightly higher than for whole leaves.

Carbon Dioxide Effects on Nitrogen

Atmospheric CO₂ concentration had no effect on leaf N concentration of sorghum but reduced leaf N of soybean (Table 3). Elevated CO₂ increased total dry matter production of sorghum 14.4% and soybean 23.9% (Table 3). The increased carbon (C) fixation and resultant dry matter production was not sufficient to alter the C:N ratio of sorghum leaf tissue but enriched CO₂ increased leaf disk C:N ratio of soybean 39% (Table 3). This increased growth apparently diluted the concentration of N in leaf tissue in soybean. The crop species x CO₂ interactions for leaf N and C:N ratio agree with greenhouse results reported by Wong (1979) who found that elevated CO₂ reduced N concentration in cotton, a C3 species, but not in corn, a C4 species. The reduction was attributed to the greater increase in dry matter for the C3 species than for the C4 species under enriched CO₂ atmosphere. Similar results were reported for wheat (*Triticum aestivum* L.), a C3 species, versus corn in a short term greenhouse study (Hocking and Meyer, 1991).

Leaf Chlorophyll Measurements

Chamber Effects: Chlorophyll meter measurements of sorghum leaves were higher than for soybean leaves. Sorghum averaged 45.2 SPAD units (the meter reads output in dimensionless units named for the acronym Soil Plant Analysis Development) and soybean averaged 40.2 SPAD units (LSD_{0.05} = 2.03). The higher meter readings for sorghum compared to soybean may have been partially related to the thicker leaves of sorghum compared to soybean. The chlorophyll meter measurement is calculated by a microprocessor based on the amount of light transmitted through a leaf from two light emitting diodes (LEDs). Thicker leaves result in reduced transmittance but higher meter readings. Specific leaf weight averaged 30.98 g/m² for sorghum and 27.50 g/m² for soybean ($P \leq 0.13$), but regression analysis showed no clear relationship between meter readings and

TABLE 3. Effect of CO₂ concentration on leaf N, leaf C: N ratio, chlorophyll concentration, chlorophyll meter reading (SPAD units), total dry matter production and seed yield of sorghum and soybean.

Crop	CO ₂ ($\mu\text{mol mol}^{-1}$)	Leaf N (g kg^{-1})	Leaf C:N -	Total Chlorophyll ($\mu\text{g cm}^{-2}$)	SPAD -	Total Dry Matter (kg ha^{-1})	Seed Yield (kg ha^{-1})	Specific Leaf Weight (g m^{-2})
Sorghum	357	36.6 a	14.0 a	38.5 a	46.6 a	7381 a	3802 b	31.07 a
	705	36.3 a	14.1 a	38.1 a	46.8 a	8676 a	4466 a	30.75 a
Soybean	357	54.5 a	9.2 b	36.1 a	40.0 a	8170 b	2501 b	29.11 b
	705	39.1 b	12.8 a	37.2 a	40.5 a	10122 a	3370 a	39.87 a
LSD ^z _{0.05}		3.28	0.48	ns ($P \leq 0.63$)	ns ($P \leq 0.87$)	ns ($P \leq 0.34$)	ns ($P \leq 0.33$)	3.93

^z LSD for species x CO₂ interaction (any two means), ns=not significant. Means within a species followed by same letter are not significantly different.

specific leaf weight. There was a trend ($P \leq 0.07$) for a species X chamber interaction in that meter readings of sorghum, but not soybean, were increased in chambers as compared to the open (Table 2).

Total chlorophyll on an area basis (chlorophyll *a* and *b*) of soybean was reduced by the chambers (Table 2). Regression analysis showed no clear relationship between chlorophyll meter readings and extractable chlorophyll. This was likely due to the narrow range of meter readings (the coefficient of variation for meter readings was only 2.6%) and the confounding effect of leaf thickness and anatomy (species variation) interactions with treatments.

Carbon Dioxide Effects: Atmospheric CO₂ concentration had no effect on chlorophyll meter readings or extractable chlorophyll of either species (Table 3). Wong (1979) in a short-term greenhouse study reported no effect of enriched CO₂ on extractable chlorophyll per unit leaf area of cotton and corn. However, other greenhouse studies have shown a reduction in extractable chlorophyll concentration with elevated CO₂ (Patterson and Flint, 1982; Wullschleger et al., 1992). The only report to date using the chlorophyll meter in a study with elevated CO₂ found that elevated CO₂ raised meter readings 7 to 8% in field-grown cotton (Pinter et al., 1994). The authors found considerable temporal variation in readings, however, and readings were significantly higher in only 11 of 27 days measured.

Elevated CO₂ increased specific leaf weight of soybean but not sorghum (Table 3). Thomas and Harvey (1983) also reported increased leaf thickness as a result of elevated CO₂ for three C3 species but not for corn, a C4 species. The C3 species¹ (soybean) increase in leaf thickness (specific leaf weight) with elevated CO₂ is counter to this species¹ response to CO₂ on leaf N (Table 3). Opposite responses to these two factors in soybean may have nullified the ability of the chlorophyll meter to serve as an estimator of leaf N concentration.

Dry Matter Production and Seed Yield

Chamber Effects: Total dry matter production was reduced 6.8% in sorghum and 12.0% in soybean by the chambers (Table 2). This resulted in a significant species X chamber interaction. Seed yield followed a similar trend. Soybean seed yield was reduced 19% by chambers, while sorghum grain yield was only reduced 9.2%.

Carbon Dioxide Effects: Elevated CO₂ increased dry matter production of sorghum 13.5% and soybean 23.8%. Separate analyses of variance by species indicated the increase was significant for soybean but not for sorghum, however, there was no significant species X CO₂ interaction (Table 3). A similar effect occurred for seed yield except that the increase was significantly greater for both species. Seed yield of sorghum increased 17.5% and soybean increased 34.7% with elevated CO₂. Although the increased yield with elevated CO₂ was more dramatic for soybean than for sorghum, the effect was not statistically different between species. These yield results agree with previous reports that the relative increase in plant growth of C3 species as compared to C4 species with elevated CO₂ atmospheres is generally greater (Wong et al., 1979; Patterson and Flint, 1980; Rogers et al., 1983a). The lack of a statistical interaction between crop species and CO₂ treatment for dry matter and seed yield, despite such a difference in response by the two crop species, points out the difficulties of doing this type work in a field situation. The technical requirements and accompanying expense of field scale CO₂ experiments limits replication while greenhouse and growth chamber studies impose artificial environmental conditions which can alter plant response to CO₂.

Both dry matter and seed yields from elevated CO₂ were greater than yields from fully-open plots (no-chamber). Since dry matter and seed yields of both crops were reduced by the artificial conditions imposed by the open-top chambers (Table 2), it is reasonable to assume that the full yield potential of these crops grown under elevated CO₂ conditions was not met due to the negative effect of the chambers.

IMPLICATIONS AND SUMMARY

Greenhouse and growth chamber studies have reported reductions in concentrations of nutrient elements other than N in plant tissue due to increased biomass production with elevated CO₂ (Peet et al., 1986; Overdieck, 1993). We found no reduction in nutrient element concentrations, other than N in soybean as a result of elevated CO₂. Our study provided a more natural environment, i.e. not a growth chamber or glasshouse environment with restricted rooting conditions. Under our more natural field-type experimental conditions, it is likely that the dilution effect

of increased biomass on plant tissue nutrient concentrations as a result of an enriched CO₂ atmosphere was offset by increased root development and nutrient uptake. Rogers et al. (1992) reported that under field conditions, cotton root development was enhanced by elevated CO₂.

Elevated CO₂, however, reduced leaf disk N 28% in soybean, the C3 species. The reduction was to a level slightly below the sufficiency range for soybean at this sampling period (Jones et al., 1991). Despite the reduction in leaf N concentration in soybean, the greatest yield response to CO₂ was with this species. This suggests that N was not limiting. Hocking and Meyer (1991) in a short-tam greenhouse study with wheat (C3 species), over a range of N rates at two levels of CO₂, found the critical concentration of N (defined as the concentration which produced 90% maximum yield) in leaf/stem tissue was reduced 24% under elevated CO₂. Our results support their conclusion that current tissue test levels for diagnosis of N deficiency may be too high for future levels of atmospheric CO₂. If this is the case, then current tissue N values used for diagnostic purposes would indicate a deficiency when none existed. However, our study was not conducted over a range of N fertilizer rates, therefore critical concentrations of N could not be determined. Whether the soybean plants' N needs under an enriched CO₂ atmosphere were met by symbiotic N fixation also could not be determined. Current work is underway to determine this.

Although CO₂ had no effect on chlorophyll meter readings, plants exhibited a narrow range of chlorophyll meter readings, and within soybean, the effect of CO₂ on leaf thickness may have compromised the ability of the meter to correlate with leaf N concentration. Therefore, we cannot say if this technology would be a useful means to diagnose plant N status under elevated CO₂ conditions. It is likely that species differences, i.e. the greater response in growth of C3 species compared to C4 species, accompanied by changes in leaf morphology of C3 species under elevated CO₂, will make calibration of chlorophyll meters even more difficult than under current environmental conditions.

In summary, our results suggest that unlike previous growth chamber reports, with the exception of N, nutrient status of field grown sorghum and soybean was not greatly affected by increased atmospheric CO₂ concentration. Further work over a range of N fertilizer rates is needed under field conditions to assess the impact of elevated CO₂ on critical concentrations of N, in C3 species especially.

Also, more intensive work needs to be done to evaluate the usefulness of chlorophyll meters in determining the N status of crops as affected by CO₂.

Finally, data from greenhouse and growth chamber studies on the effect of nutrient status of plants as affected by CO₂ should be interpreted with caution as even under the large open-top chambers used in our field-scale study, the chambers exerted an effect on leaf chlorophyll concentration, tissue N, biomass production, and seed yield.

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